Reference: *Biol. Bull.*, 152: 199–208. (April, 1977)

EFFECTS OF A JUVENILE HORMONE MIMIC ON MALE AND FEMALE GAMETOGENESIS OF THE MUD-CRAB, *RHITHROPANOPEUS HARRISII* (GOULD) (BRACHYURA: XANTHIDAE)

GENEVIEVE G. PAYEN[1] AND JOHN D. COSTLOW

Duke University Marine Laboratory, Beaufort, North Carolina 28516

Juvenile hormone (JH) analogs are substances of natural and synthetic origin which act in the same way as endogenous JH, the endocrine secretion of the insect *corpora allata*. The wide range of their biological effects is now well established and has led to their study as a possible means of pest control (reviews by Novák, 1971; Stockel, 1975). In consequence, nontarget organisms may also be contaminated through the food-chain or by residues of the exogenous hormone degradation; likewise, JH analogs may enter the aquatic surroundings with land-drainage or erosion from the adjacent pesticide-treated agricultural lands and tidal marshes. Considering the potential role played by JH analogs as insecticides, it seems of particular interest to determine whether these compounds may affect crustaceans, generally accepted to be closely related to insects.

JH mimics are known to disrupt embryonic and larval insect development and to induce sterility in adults (reviews by Bowers, 1971; Sláma, 1971; Sláma, Romaňuk and Sŏrm, 1974), rather than to cause immediate death. However, the results depend largely on the type of analog, the dose and time of application and also the optimal sensitive period of the animal. Although there have not been sufficient investigations to demonstrate the existence of crustacean organs similar to the insect *corpora allata*, one must take into consideration the recent ultrastructural study by Byard and Shivers (1975), which suggests a possible analogy with decapod mandibular organs. In line with this hypothesis, decapods thus appear to be a good choice to search for target organs which can respond to insect JH. Knowledge of the normal reproductive physiology of the estuarine mud-crab *Rhithropanopeus harrisii* (Payen, 1974b) and the availability of one JH mimic (Altosid®) prompted this investigation, to determine in what way male and female gametogenesis in an estuarine crab may be affected under laboratory conditions.

Very few studies have been devoted to the influence of natural or synthetic JH on crustaceans. A physiological effect showing vitellogenic inhibition has been observed in the amphipod *Orchestia gammarellus* (Charniaux-Cotton, 1974) after injection of synthetic JH-I. Two morphological effects are actually known. One is related to the blocking of egg development in the isopod *Armadillidium vulgare* (Greer, cited in Pihan, 1975) with topical application of both analogs and natural JH-I; the other demonstrates a precocious metamorphosis without settlement in the acorn barnacle, *Balanus galeatus*, reared with the synthetic analog,

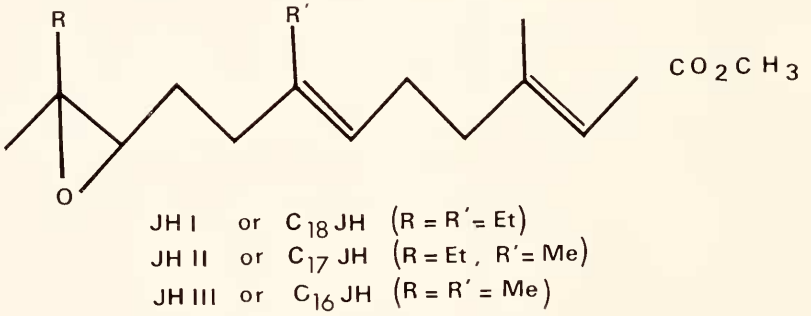
[1] Present address: Université Pierre et Marie Curie, Laboratoire Sexualité et Reproduction des Invertébrés, Bâtiment A, 4 Place Jussieu, 75230 Paris Cédex 05, France.

ZR-512 (Gomez, Faulkner, Newman and Ireland, 1973) or with freshly synthesized JH-I (Ramenofsky, Faulkner and Ireland, 1974). A third study (Costlow, 1976) has shown that a similar compound, methoprene (Altosid®: ZR-515), while not affecting metamorphosis itself, does result in reduced survival of larvae of the mud-crab, *Rhithropanopeus harrisii;* there is evidence of synergistic effects of salinity and methoprene, and also that the early megalopa, as opposed to the zoeal stages, represents a developmental stage of extreme sensitivity.

Material and Methods

Hormonal material

Numerous chemical compounds with juvenilizing activity, also called juvenoids or insect growth regulators, have been synthesized during the past few years (reviews by Sláma, Romañuk and Sŏrm, 1974; Pihan 1975). We used methoprene (made by the Zoëcon Corporation, Palo Alto, California), or isopropyl-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate or Altosid® (ZR-515), which is relatively stable. Its efficacy has been proved in different insects: *Galleria mellonella, Aedes aegypti, Tenebrio molitor, Schistocerca gregaria, Culex tarsalis, Gryllus bimaculatus* and *Musca domestica* (Henrick, Staal, Siddal, 1973; Reddy and Krishnakumaran, 1973; Roussel and Perron, 1974; Ittycheriah, Marks and Quraishi, 1974; Crochard, 1975; Yu and Terriere, 1975). This $C_{19}H_{34}O_3$ compound is a mixture of isomers containing 69.0% *trans trans* form, closely related



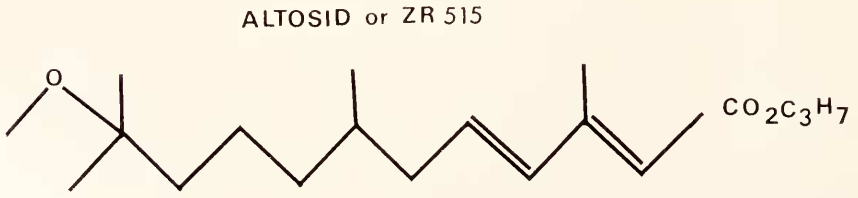


Figure 1. Comparative chemical structures of the acids of the three juvenile hormones known in insects (JH-I, JH-II, JH-III) and one analog, methoprene (Altosid® or ZR-515). *Cis* and *trans* configurations are omitted in the diagrams.

to the three juvenile hormones (JH-I, JH-II, JH-III) now known to occur in nature (Fig. 1). Its chromatographic properties have been recently analyzed by Dunham, Schooley and Siddall (1975).

Experimental animals

In August 1974, 28 mature specimens of *Rhithropanopeus harrisii* (Gould) (14 females, 14 males) with a cephalothoracic length varying between 5 and 8 mm were collected in the Neuse River estuary at a place called "Pine Cliff" near Havelock, North Carolina, and kept isolated.

The experimental series included seven male and seven female crabs which were reared in 1.30 ppm Altosid® (90.7% active ingredient) in 20‰ sea water at 25° C. Methoprene was dissolved in 100 ml of full strength insecticide grade acetone to make a stock solution. A dilution of 1.30 ppm was made by adding 1 ml of the stock solution to 999 ml of 20‰ sea water. This concentration is close to the limit (1.39 ppm) of solubility of methoprene and allows one to determine the effect of the highest concentration of the compound.

There were two control series: a seawater control with three crabs of each sex and an acetone control with four crabs of each sex. The seawater control had a salinity of 20‰, and the acetone control was made up by adding 1 ml of full strength acetone to 999 ml of sea water with a salinity of 20‰. Thus, the acetone control and the experimental medium had the same concentration of 1 ppt acetone. The crabs were changed every three days to clean bowls containing fresh media and fed with four day old *Artemia salina* nauplii.

No morphological or physiological differences were noted between the crabs reared in seawater control and acetone control. Three females laid eggs in these media, but none were laid in the experimental one. The whole experiment was conducted for one and a half months.

Methoprene activity was assayed on *R. harrisii* spermatogenesis and oogenesis following a double method of application: ingestion and topical application in the ambient aquatic environment. This process is actually considered to be less toxic and more active than injections (Sláma, Romaňuk and Sŏrm, 1974, pp. 98–104).

Histological procedure

Testes and ovaries were examined both anatomically and histologically. Whole mounts and 5 μm sections of treated and control gonads were prepared after being removed from the crabs and fixed in Duboscq Brasil's solution. Alum-carmine and haematoxylin-picro-indigo carmine were the primary stains. Study of *R. harrisii* gonads was undertaken on crabs 4.7 to 8.0 mm cephalothoracic length. To follow the appearance of possible abnormalities or gametogenic perturbations which take place after methoprene treatment, the animals were sacrificed after 12 and 45 days.

Results

Although no particular relationship between physiological processes such as molting and gametogenesis is known, it seems appropriate to include the following observations for future studies.

TABLE I

Summary of seven male and seven female experimental crabs, their molt status, and times of exposure to methoprene.

Status of crabs at examination			Number of days of exposure to methoprene (1.39 ppm)	Size: cephalo-thoracic length (mm)	Effects on gametogenesis
Without molting	Just after a lethal molt	Three days after a normal molt			
♂			7	5.0	stimulation of spermat-ogenesis
♂			9	6.2	
♂			12	7.0	
♂			24	8.0	inhibition of spermato-genesis
	♂		43	7.2	
	♂		43	4.9	
		♂	45	8.0	
♀			15	5.3	no effect
♀			15	6.6	inhibition of vitello-genesis
♀			18	6.9	
	♀		38	6.8	
	♀		41	7.0	
	♀		42	7.5	
♀			45	7.3	

Two females and one male out of six reared in sea water molted during the experimental period. Among those maintained in the sea water-acetone medium, one male and two females molted normally, whereas one female died 18 hours after exuviation. Crabs from the experimental series (sea water plus methoprene-acetone solution) showed a high rate of mortality at the time of ecdysis which always appeared delayed (between 38 to 43 days of treatment) compared to controls of the same size (average of eight days after collecting). Only one male survived after shedding its exuvia, whereas this phase was lethal for two other males and three females. Nonachievement of the molting process thus appears to be due to the combined effects of both methoprene and acetone, which may produce a dissolving action through the new formed integument. Table I summarizes the data obtained.

Effect of Altosid® on R. harrisii *spermatogenesis*

In previous work (Payen, 1974a), a correlation between the encasement of gonopods (Pl_2 becomes inserted into a foramen of Pl_1 instead of remaining free and independent) and the onset of spermatogenesis was found, allowing a quick evaluation of the state of the gonads, especially in young males. Only crabs with encased gonopods were considered. After methoprene treatment, they all exhibited a more developed genital apparatus than that of controls with the same cephalothoracic length but were less developed than crabs destalked during larval life and sacrificed at juvenile stages (Payen, Costlow and Charniaux-Cotton, 1971).

In particular, the androgenic glands, which are known to be affected by destalking experiments (Payen, 1974b), always keep a volume and a structure similar to those of control crabs. During the first twelve days, all stages of spermatogenesis are well recognizable; they take place in more numerous and prominent convolutions than in testes of animals reared only in sea water. Moreover, the genital ducts are swollen and full of homogenous acidophilic secretions. In males sacrificed between 18 and 45 days, the testes appear stunted and become difficult to dissect (60 μm of average diameter as opposed to 120 μm in males of the same size reared in seawater control). Spermatogenesis is considerably reduced; most of the testicular volume is taken up by rare spermatocytes in meiotic prophase, few spermatids and many spermatozoa which are drained off in the overstretched spermducts. Gonia disappear, and the few which are left do not show mitotic figures as in control testes; some are pycnotic. Since cell divisions are approximately synchronous within each testicular lobe, it is easy to note the amalgam of spermatocyte clusters which constitutes degenerative glomerules reaching a diameter of about 15 μm; these compact formations are more or less scattered or abundant, depending on time of sacrifice. Thus, the ultimate step of degeneration leads to the complete lysis of the testicular lobes. It then appears that the methoprene-exposed crabs undergo a transitory increase of spermatogenesis (during the first 12 days of contamination), characterized by a massive accumulation of spermatozoa in the *vasa deferentia*. Later, no new spermatogenesis occurs, because spermatogonial mitoses and spermatocyte meiosis progressively stop. We have never observed any inhibition of the draining of spermatozoa in the spermducts as was described for two species of locust under the action of an excess of JH during larval instars (Cantacuzène, 1968).

Effect of Altosid® on R. harrisii *oogenesis*

Ovaries of young females, exposed to JH mimic and sacrificed at a cephalothoracic length which does not go beyond 5.5 mm, present a morphology similar to ovaries of crabs of the same size reared in control media. As already described, ovaries of normal crabs (Payen, 1974a) exhibit an internal region containing gonia and oocytes with conspicuous chromosomes and, located at the periphery of the gonad, strands of oocytes in previtellogenic growth enclosed by follicle cells. No vitellogenic oocyte could be detected in ovaries of either exposed or nonexposed females. Methoprene, therefore, does not seem to affect the beginning of oogenesis, which occurs as normally as in controls.

But the situation is quite different among treated females of a cephalothoracic length over 6 mm. As opposed to those reared in control media, which essentially show darkly-pigmented oocytes ranging in diameter from 130 to 220 μm, no fully-developed oocyte can be seen in ovaries of females sacrificed after 30 days of exposure. The milky aspect of the gonads, the light acidophilic affinity of the oocyte cytoplasm and the average diameter of 85 μm reveal the incapacity to achieve vietellogenesis. After 15 days of exposure, besides gonia and oocytes with conspicuous chromosomes, some pigmented oocytes are still present; while in numerous others, further development appears blocked at terminal previtellogenesis (40 μm of average diameter) or at an early vitellogenic phase. This ultimate effect

on oogenesis appears clearly in crabs sacrificed after 30 days and shows the strong effect of a JH-like compound on yolk deposition.

The presence of few pigmented oocytes in early sacrificed females can be explained by the fact that oocytes already engaged in vitellogenesis at the beginning of the experiment are able to go through the whole phenomenon without being disturbed by methoprene application. After a longer experimental period, the complete disappearance of pigmented oocytes seems due to their release in the oviducts, although no real spawning could be detected. However, also noticed in the ovaries was a rather high degree of resorption both of these oocytes, and of younger ones blocked in early vitellogenesis, by the proliferating follicle cells.

Discussion

Study of female and male gametogenesis of the crab *R. harrisii* reared in the laboratory in the presence of methoprene indicates that this JH mimic acts as a chemosterilant which induces a progressive inhibition of vitellogenesis and stimulation of spermatogenesis after a short time of exposure (12–15 days), and a pronounced inhibition of both spermatogenesis and vitellogenesis after a longer delay.

Oosorption obtained after methoprene treatment is similar to the effect occurring in normal and destalked females which have experimentally received two androgenic glands (Payen, 1969, 1975). Likewise, a transitory increase of testes development, due to an androgenic hyperactivity (Payen, Costlow, and Charniaux-Cotton, 1971) preceding degeneracy, has been observed in juvenile crabs deprived of eyestalks (Payen, unpublished). Thus, after a longer experimental delay, an excess of androgenic hormone can affect the maintenance of spermatogenesis as methoprene does.

Few results concern the effects of compounds with JH activity on insect male physiology. Besides sexual behavior, it is known that endogenous JH is required for the regulation of spermatophore production in *Hyalophora cecropia* accessory glands (Williams, 1956), as well as tubular gland-ejaculatory duct and accessory gland enlargement in *Nymphalis antiopa* (Herman and Bennett, 1975). In most insect species, spermatogenesis takes place at nymphal stage and continues during the postlarval life in the presumed absence of the molting hormone (ecdysone) and presence of JH (Engelmann, 1970, p. 191; Dumser and Davey, 1974). Knowledge of the role of the latter hormone, or its mimics, in development and function of the testes is, so far, very limited. Economopoulos and Gordon (1971) did not find any modification of spermatocyte differentiation in nymphs of *Oncopeltus fasciatus* by treatment with JH analogs. Landa and Metwally (1974), using two JH analogs on pupae of the Coleoptera, mentioned the persistence of a normal spermatogenesis with one analog (Ethyl 11-chloro-3, 7, 11 trimethyl 1-2 dodecenoate), whereas a certain perturbation of the initial and middle phases of the male gametogenesis happens with the other applied analog (6, 7-epoxygeranyl 3, 4-methylendioxyphenyl). Dumser and Davey (1974) reported that farnesyl methyl ether (JH analog) produces a decline of the rate of spermatogonial mitosis and consequently a curtailment of meiotic activity on *Rhodnius prolixus*. More recently, Szöllösi (1975) observed an inhibition of the imaginal differentiation of the spermducts of two acridids but no direct effect on spermatogenesis after injec-

tion of either a synthetic Cecropia hormone or an analog (hydroprene or ZR-512) during late larval life. According to Reissig and Kamm (1975), the synthetic analog methyl 10, 11-epoxy-7-ethyl-3, 11 dimethyl-2, 6-tridecadienoate does not act on growth and development of the testes of *Draeculacephala crassicornis* in diapause, but hastens initial sperm maturation. While allatectomy or implantation of supernumerary *corpora allata* have provided evidence which implicates JH in the inhibition of testicular development (review by Dumser and Davey, 1974, page 1020), the few conflicting results obtained after treatment with JH mimics actually prevent one from drawing a similar conclusion.

In contrast to males, many studies have shown a gonadotropic effect of JH or juvenoids in adult females of several insect species (reviews by Engelmann, 1970; Sláma, Romañuk and Sõrm, 1974; Herman and Bennett, 1975). With a few exceptions, such as *Bombyx mori* (Bounhiol, 1942), initiation of vitellogenesis appears to be the result of the secretory activity of both the *pars intercerebralis*, located in the protocerebron, and the *corpora allata*. JH secretion, or juvenoid application, stimulates vitellogenin (female-specific protein yolk precursor) synthesis, its release from the fat body, and its incorporation into the oocytes through the follicle cells (Bell and Barth, 1971; Wyss-Huber and Lüscher, 1972; Davey and Huebner, 1974; Abu-Hakima and Davey, 1975). Response to juvenoid administration during adult life leads to anomalies or inhibition of embryonic development (reviews by Patterson, 1974; Pihan, 1975).

In decapods, it is known that spermatogenesis only takes place during the postlarval life (Payen, 1974a). Considering the fact that most of these malacostracans can molt during their whole life, male gametogenesis may occur when molting hormone is present. Although we have yet no information on the possible presence of other endocrine substances such as JH, it is remarkable to compare the similarity of our results with those relating the modifications of the respiratory and energetic metabolism of two noxious *Lepidoptera* treated with a synthetic insecticide (DU 19111) applied from the fourth larval instar (Moreau, Castex and Lamy, 1975). Initial stimulation of the metabolism followed by an important depression recalls, indeed, the phenomena which take place in the testes of *R. harrisii* treated with methoprene.

Concerning the mechanism of oocyte lysis, it is interesting to note that an analogy can be established with the amphipod *Orchestia gammarellus* after injection of synthetic JH-I (Charniaux-Cotton, 1974). Thus, methoprene and JH-I appear to act in the same inhibitory way on vitellogenesis of both amphipods and decapods; whereas, as indicated at the beginning of this work, these types of compounds are usually responsible for promoting a precocious growth of oocytes by deposition of yolk in most insect species (compare with Sláma, Romañuk and Sõrm, 1974, pp. 36–39 and 236–243). However, resorption of vitellogenic oocytes has been observed in reproducing females of *Thysanura*, the firebrat *Thermobia domestica* (Rohdendorf and Sehnal, 1973; Rohdendorf, 1975) following application of various juvenoids. Malacostracan crustaceans and aptergygote insects show a close relation in that they maintain the capacity to ecdyse throughout postlarval life. It, then, becomes understandable why a pterygote insect imago differs from a mature apterygote or a crustacean: in this latter arthropod, the molting glands (prothoracic or so-called ventral glands in insects and Y-organs in crustaceans)

function during the whole life, whereas such organs would degenerate in most pterygotes as soon as metamorphosis is completed. From this different physiological situation, crustacean postlarval stages appear more comparable with the ptergygote larvae than their imago.

If the alterations of male and female gametogenesis are true effects, *i.e.*, really due to the hormone-like compound and not to a toxic reaction, there is no doubt that the observed results suggest certain analogies between juvenile and androgenic hormones, since both of them affect male and female gametogenesis in the same way. In fact, it appears that the ingested and topically applied JH analog is either working with an endogenous crustacean hormone closely related to insect JH or outcompeting it for the same active site. The exogenous compound may also be chemically modified to an androgenic-like hormone once it reaches the hemolymph. As already suggested above, the synthetic hormone used in the present work is neither identical or a likely precursor of the natural insect hormone but possesses the juvenilizing as well as the gonadotropic effect. Moreover, the tested concentration (1.30 ppm) probably far exceeds normal physiological doses. It is interesting to point out that no larvae of the same species survived beyond the first zoeal stage at 1.00 ppm ZR-515 under optimum or under stress conditions of salinity and temperature (Costlow, 1976; Christiansen, Costlow and Monroe, 1977).

The availability of various synthetic and natural insect hormones may provide a better understanding of arthropod physiology. For example, more experiments involving destruction or ablation of Y-organs in crabs exposed to a JH analog could complete our findings and bring new data concerning the factors required for vitellogenesis and spermatogenesis in malacostracans. The lack of the source of ecdysone would then allow an easier comparison of the results in pterygote insects and crustaceans.

Portions of the work were supported by a contract between the Office of Naval Research and Duke University, NR 104–194. Thanks are due to Professor H. Charniaux-Cotton for helpful comments on the manuscript.

Summary

1. Effects of ingestion and topical application of 1.30 ppm methoprene, an insect juvenile hormone (JH) mimic, include gametogenesis disorders in both male and female mud-crab *Rhithropanopeus harrisii*.

2. Progressive inhibition of vitellogenesis and stimulation of spermatogenesis take place after a short exposure time (12–15 days), whereas a longer period (between 30 to 45 days) promotes inhibition of both spermatogenesis and vitellogenesis. Such modifications demonstrate the chemosterilizing action of methoprene on crustaceans.

3. Stoppage of vitellogenesis is characterized by a blockage of oocytes at terminal previtellogenesis and proliferation of the follicle cells involved in vitellogenic oocyte lysis. Spermatogenic degeneration begins with the amalgam of spermatocyte clusters in meiotic prophase and pycnosis of gonia.

4. Analogies between these results and those obtained from experiments involving androgenic gland implantations or the consequences of their hyperfunctioning are discussed.

LITERATURE CITED

Abu-Hakima, R., and K. G. Davey, 1975. Two actions of juvenile hormone on the follicle cells of *Rhodnius prolixus* Stal. *Can. J. Zool.*, **53**: 1187–1188.

Bell, W. J., and R. H. Barth Jr., 1971. Initiation of yolk deposition by juvenile hormone. *Nature, New Biol.*, **230**: 220–222.

Bounhiol, J. J., 1942. L'ablation des corps allates au dernier âge larvaire n'affecte pas la reproduction chez *Bombyx mori*. *C. R. Hebd. Seances Acad. Sci. Paris Ser. D Sci. Nat.* **215**: 234–236.

Bowers, W. S., 1971. Juvenile hormones. Pages 307–332 in M. Jacobson and D. G. Crosby, Eds., *Naturally occurring insecticides*. M. Dekker, New York.

Byard, E. H., and R. R. Shivers, 1975. The mandibular organ of Lobster, *Homarus americanus*. *Cell. Tissue Res.*, **162**: 13–22.

Cantacuzène, A. M., 1968. Différenciation des caractères sexuels mâles de deux Acridiens après implantation des corps allates pendant les derniers stades larvaires. *C. R. Hebd. Seances Acad. Sci. Paris Ser. D Sci. Nat.*, **267**: 749–752.

Charniaux-Cotton, H., 1974. Données nouvelles concernant la vitellogenèse des Crustacés Malacostracés obtenues chez l'Amphipode *Orchestia gammarellus* (Pallas); folliculogenèse à partir d'un tissu permanent; action du busulfan; action inhibitrice de l'hormone juvénile. *C. R. Hebd. Seances Acad. Sci. Paris Ser. D Sci. Nat.*, **279**: 563–566.

Christiansen, M. E., J. D. Costlow, Jr., and R. J. Monroe, 1977. Effect of the juvenile hormone mimic ZR-515 (Altosid®) on the larval development of the mud-crab *Rhithropanopeus harrisii* in various salinities and cyclic temperatures. *Mar. Biol.*, **39**: in press.

Costlow, J. D., Jr., 1976. The effect of the juvenile hormone mimics on development of the mud-crab, *Rhithropanopeus harrisii* (Gould). Pages 439–459 in A. Calabrese, F. P. Thurberg, and F. J. Vernberg, Eds., *Proc. symp. on pollution and physiology of marine organisms*. Academic Press, New York.

Crochard, C., 1975. Définition du test "Grillon" d'activité ovidice des analogues de l'hormone juvénile des insectes. *C. R. Hebd. Seances Acad. Sci. Paris Ser. D. Sci. Nat.*, **281**: 61–64.

Davey, K. G., and E. Huebner, 1974. The response of the follicle cells of *Rhodnius prolixus* to juvenile hormone and antigonadotropin *in vitro*. *Can. J. Zool.*, **52**: 1407–1412.

Dumser, J. B., and K. G. Davey, 1974. Endocrinological and other factors influencing testis development in *Rhodnius prolixus*. *Can. J. Zool.*, **52**: 1011–1022.

Dunham, L. L., D. A. Schooley, and J. B. Siddall, 1975. A survey of the chromatographic analysis of natural insect juvenile hormones and the insect growth regulator, Altosid®. *J. Chromatogr. Sci.*, **13**: 334–336.

Economopoulos, A. P., and H. T. Gordon, 1971. Growth and differentiation of testes in the large milkweed bug, *Oncopeltus fasciatus* (Dallas). *J. Exp. Zool.*, **177**: 391–405.

Engelmann, F., 1970. *The physiology of insect reproduction*. International Series of monographs in pure and applied biology, Vol. 44. Zoology division, Pergamon Press, Oxford, 307 pp.

Gomez, E. D., D. J. Faulkner, W. A. Newman, and C. Ireland, 1973. Juvenile hormone mimics: effect on cirriped crustacean metamorphosis. *Science*, **179**: 813–814.

Henrick, C. A., G. B. Staal, and J. B. Siddall, 1973. Alkyl 3, 7, 11-trimethyl-2, 4 dodecodienoates, a new class of potent insect growth regulators with juvenile hormone activity. *J. Agr. Food Chem.*, **21**: 354–359.

Herman, W. S., and D. C. Bennet, 1975. Regulation of oogenesis, female specific protein production, and male and female reproductive gland development by juvenile hormone in the butterfly, *Nymphalis antiopa*. *J. Comp. Physiol.*, **99**: 331–338.

Ittycheriah, P. I., E. P. Marks, and M. S. Quraishi, 1974. Effects of hormones, brain, and endocrine glands on the ovary of *Culex tarsalis in vivo* and *in vitro*. *Ann. Entomol. Soc. Am.*, **67**: 595–600.

Landa, V., and M. M. Metwally, 1974. Effects of conventional chemosterilants and juvenile hormone analogues on spermatogenesis in *Trogoderma granarium* (Coleoptera, Dermestidae). *Acta Entomol. Bohemoslov.*, **71**: 145–152.

Moreau, R., C. Castex, and M. Lamy, 1975. Examen préliminarie de quelques aspects des effets métaboliques d'un nouvel insecticide de synthèse chez deux insectes nuisibles:

Pieris brassicae L. et *Thaumetopoea pityocampa* S. (Lépidoptères). *Ann. Zool. Ecol. Anim.*, **7**: 161–170.

Novák, V. J. A., 1971. Juvenile hormone analogues and their theoretical and practical significance. *Acta Phytopathol. Acad. Sci. Hung.*, **6**: 203–210.

Patterson, J. W., 1974. A comparison of the morphogenetic and sterilizing activities of Juvenile Hormone mimics on *Aedes aegypti*. *J. Insect. Physiol.*, **20**: 2095–2106.

Payen, G., 1969. Expériences de greffes de glandes androgènes chez la femelle pubère du Crabe *Rhithropanopeus harrisii* (Gould) (Crustacé, Décapode). *C. R. Hebd. Seances Acad. Sci. Paris Scr. D Sci. Nat.*, **268**: 393–396.

Payen, G., 1974a. Morphogenèse sexuelle de quelques Brachyoures (Cyclométopes) au cours du développement embryonnaire, larvaire et postlarvaire. *Bull. Mus. Natl. Hist. Nat. Paris Scr. 3, 209 Zool.*, **139**: 201–262.

Payen, G., 1974b. Recherches sur la réalisation et le contrôle de la différenciation sexuelle chez les Crustacés Décapodes Reptantia. *Thèse d'Etat*, Paris, AO, CNRS-9930, 224 pp.

Payen, G., 1975. Effets masculinisants des glandes androgènes implantées chez la femelle pubère pédonculectomisée de *Rhithropanopeus harrisii* (Gould) (Crustacé Décapode Brachyoure). *C. R. Hebd. Seances Acad. Sci. Paris Scr. D Sci. Nat.*, **280**: 1111–1114.

Payen, G., J. D. Costlow, and H. Charniaux-Cotton, 1971. Etude comparative de l'ultrastructure des glandes androgènes de Crabes normaux et pédonculectomisés pendant la vie larvaire ou après la puberté chez les espèces *Rhithropanopeus harrisii* (Gould) et *Callinectes sapidus* Rathbun. *Gen. Comp. Endocrinol.*, **17**: 526–542.

Pihan, J. C., 1975. Utilisation des effets tératogènes des hormones juvéniles et de leurs mimétiques dans la lutte contre les insectes. *Année Biol.*, **14**: 29–44.

Ramenofsky, M., D. J. Faulkner, and C. Ireland, 1974. Effect of juvenile hormone on cirriped metamorphosis. *Biochem. Biophys. Res. Commun.*, **60**: 172–178.

Reddy, G., and A. Krishnakumaran, 1973. Changes in the morphogenetic response of *Tenebrio molitor* to juvenile hormone in relation to age. *J. Insect Physiol.*, **19**: 773–780.

Reissig, W. H., and J. A. Kamm, 1975. Effect of synthetic juvenile hormone on reproduction of adult male *Draeculacephala crassicornis*. *Ann. Entomol. Soc. Am.*, **68**: 353–354.

Rohdendorf, E. B., 1975. Histological changes in the ovaries of *Thermobia domestica* (Thysanura) following application of juvenoids. *Acta Entomol. Bohemoslov.*, **72**: 209–220.

Rohdendorf, E. B., and F. Sehnal, 1973. Inhibition of reproduction and embryogenesis in the firebrat, *Thermobia domestica* by juvenile hormone analogues. *J. Insect Physiol.*, **19**: 37–56.

Roussel, J. P., and J. M. Perron, 1974. Action de substances mimétiques de l'hormone juvénile sur *Schistocerca gregaria* Forsk. *Arch. Zool. Exp. Gén.*, **115**: 251–262.

Sláma, K., 1971. Insect juvenile hormone analogues. *Annu. Rev. Biochem.*, **40**: 1079–1102.

Sláma, K., M. Romaňuk, and Sörm, 1974. *Insect hormones and bioanalogues*. Springer-Verlag, Wien, New York, 477 pp.

Stockel, J., 1975. Les analogues de l'hormone juvénile, ou régulateurs de croissance chez l'insecte. Possibilités d'application dans la lutte contre les espèces nuisibles. *Ann. Zool. Ecol. Anim.*, **7**: 91–118.

Szöllösi, A., 1975. Imaginal differentiation of the spermiduct in acridids: effects of juvenile hormone. *Acrida*, **4**: 205–216.

Williams, C. M., 1956. The juvenile hormone of insects. *Nature*, **178**: 212–213.

Wyss-Huber, M., and M. Lüscher, 1972. *In vitro* synthesis and release of proteins by fat body and ovarian tissue of *Leucophaea maderae* during the sexual cycle. *J. Insect. Physiol.*, **18**: 689–710.

Yu, S. J., and L. C. Terriere, 1975. Microsomal metabolism of juvenile hormone analogs in the housefly, *Musca domestica* L. *Pest. Biochem. Physiol.*, **5**: 418–430.